

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): An isolated, synthetic or recombinant nucleic acid comprising

(a) a nucleic acid sequence encoding a polypeptide having pectate lyase (EC 4.2.2.2) activity having at least ~~50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, [[or]] 99%, or more, or 100%~~ sequence identity to ~~SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, or enzymatically active fragments thereof over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a gene or transcript;~~

(b) a nucleic acid sequence encoding a polypeptide having pectate lyase(EC 4.2.2.2) activity comprising the sequence SEQ ID NO:77, SEQ ID NO:131 or SEQ ID NO:133, or an enzymatically active fragment thereof;

(c) a nucleic acid sequence encoding a polypeptide having pectate lyase (EC 4.2.2.2) activity, wherein the polypeptide has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or 100% sequence identity to SEQ ID NO:78, SEQ ID NO:132 or SEQ ID NO:134, or an enzymatically active fragment thereof;

(d) a nucleic acid sequence encoding a polypeptide having pectate lyase (EC 4.2.2.2) activity, wherein the polypeptide has the sequence SEQ ID NO:78, SEQ ID NO:132 or SEQ ID NO:134, or an enzymatically active fragment thereof;

(e) the nucleic acid of (a), (b), (c) or (d) but lacking a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof;

(f) the nucleic acid of (a), (b), (c), (d), or (e) further comprising a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme; or

(g) a sequence complementary to (a), (b), (c), (d), (e), or (f)
wherein the nucleic acid encodes at least one polypeptide having a pectate lyase activity,
wherein optionally and the sequence identities are determined by analysis with a
sequence comparison algorithm or by a visual inspection.

Claims 2 to 8 (canceled)

Claim 9 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim [[8]] 1, wherein the pectate lyase activity comprises the breakup or dissolution of a plant fiber or a plant cell wall[[s]]; beta-elimination (trans-elimination) cleavage of pectin, polygalacturonic acid

(pectate), homogalacturonan, rhamnogalacturonan; 1,4-linked alpha-D-galacturonic acid, and/or methyl-esterified galacturonic acid; catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid); and/or beta-elimination (trans-elimination) cleavage of galactan to galactose or galactooligomers

Claims 10 to 15 (canceled)

Claim 16 (currently amended): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises activity the same or similar to pectate lyase (EC 4.2.2.2), ~~poly(1,4 alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC 3.2.1.82)~~.

Claims 17 to 23 (canceled)

Claim 24 (currently amended): An isolated, synthetic or recombinant nucleic acid, ~~wherein the nucleic acid comprises comprising:~~

(a) a nucleic acid sequence that hybridizes under stringent conditions to the complete complement of a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133.

wherein the nucleic acid encodes a polypeptide having a pectate lyase activity and,
wherein the stringent conditions include a wash step comprising a wash in 0.2.times SSC
at a temperature of about 65 degrees C. for about 15 minutes, or

(b) a nucleic acid sequence complementary to the sequence of (a).

Claims 25 to 26 (canceled)

Claim 27 (currently amended): A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a pectate lyase activity, wherein the probe comprises at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400 or 500 consecutive bases of a sequence comprising ~~SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133,~~
wherein the probe identifies the nucleic acid by binding or hybridization.

Claims 28 to 30 (canceled)

Claim 31 (previously presented): An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

Claims 32 to 33 (canceled)

Claim 34 (previously presented) A method of amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

Claim 35 (previously presented): An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

Claim 36 (previously presented): A vector or cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

Claims 37 to 39 (canceled)

Claim 40 (previously presented): A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

Claims 41 to 42 (canceled)

Claim 43 (previously presented): A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.

Claim 44 (canceled)

Claim 45 (previously presented): A transgenic plant or seed comprising a sequence as set forth in claim 1 or claim 24.

Claims 46 to 48 (canceled)

Claim 49 (previously presented): An antisense oligonucleotide comprising a nucleic acid

sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

Claim 50 (canceled)

Claim 51 (previously presented): A method of inhibiting the translation of a pectate lyase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

Claim 52 (previously presented): A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

Claim 53 (canceled)

Claim 54 (previously presented): A method of inhibiting the expression of a pectate lyase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.

Claim 55 (currently amended): An isolated, synthetic or recombinant polypeptide having pectate lyase (EC 4.2.2.2) activity (i) having

(a) comprising at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, [[or]] 99%, or more or 100% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ

~~ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134, over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or an enzymatically active fragment thereof:~~

(b) comprising SEQ ID NO:78, SEQ ID NO:132 or SEQ ID NO:134;

(c) encoded by a nucleic acid having a sequence of claim 1;

(d) the polypeptide of (a), (b), or (c), but lacking a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof; or

(e) the polypeptide of (a), (b), (c), or (d), further comprising a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme;

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, ~~or, (ii) encoded by a nucleic acid having at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID~~

~~NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131, SEQ ID NO:133.~~

Claims 56 to 60 (canceled)

Claim 61 (currently amended) The isolated, synthetic or recombinant polypeptide of claim 60, wherein the pectate lyase activity comprises the breakup or dissolution of a plant fiber or a plant cell wall[[s]]; beta-elimination (trans-elimination) cleavage of pectin, polygalacturonic acid

(pectate), homogalacturonan, rhamnogalacturonan; 1,4-linked alpha-D-galacturonic acid, and/or methyl-esterified galacturonic acid; catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid); and/or beta-elimination (trans-elimination) cleavage of galactan to galactose or galactooligomers.

Claims 62 to 67 (canceled)

Claim 68 (currently amended) The isolated, synthetic or recombinant polypeptide of claim 59, wherein the pectate lyase activity comprises activity the same or similar to pectate lyase (EC 4.2.2.2), ~~poly(1,4 alpha-D-galacturonide) lyase (EC 4.2.2.2)~~, ~~polygalacturonate lyase (EC 4.2.2.2)~~, ~~pectin lyase (EC 4.2.2.10)~~, ~~polygalacturonase (EC 3.2.1.15)~~, ~~exo-polygalacturonase (EC 3.2.1.67)~~, ~~exo-polygalacturonate lyase (EC 4.2.2.9)~~ or ~~exo-poly-alpha-galacturonosidase (EC 3.2.1.82)~~.

Claims 69 to 80 (canceled)

Claim 81 (currently amended) The isolated, synthetic or recombinant polypeptide of claim 55, wherein the polypeptide comprises at least one glycosylation site.

Claims 82 to 85 (canceled)

Claim 86 (previously presented): A protein preparation comprising a polypeptide as set forth in claim 55, wherein the protein preparation comprises a liquid, a solid or a gel.

Claim 87 (previously presented): A heterodimer comprising a polypeptide as set forth in claim 55 and a second domain, or, a homodimer comprising a polypeptide as set forth in claim 52.

Claims 88 to 90 (canceled)

Claim 91 (previously presented): An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 55, or a subsequence thereof.

Claim 92 (canceled)

Claim 93 (previously presented): An array comprising an immobilized polypeptide as set forth in claim 55.

Claim 94 (previously presented): An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.

Claim 95 (currently amended): An isolated, synthetic or recombinant antibody that specifically binds to a polypeptide as set forth in claim 55.

Claim 96 (canceled)

Claim 97 (previously presented): A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 55.

Claim 98 (previously presented): A method of isolating or identifying a polypeptide with a pectate lyase activity comprising the steps of:

(a) providing an antibody as set forth in claim 95;
(b) providing a sample comprising polypeptides; and
(c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a pectate lyase activity.

Claim 99 (previously presented): A method of making an anti-pectate lyase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24, or a polypeptide as set forth in claim 55, or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-pectate lyase antibody.

Claim 100 (canceled)

Claim 101 (previously presented): A method of producing a recombinant polypeptide comprising the steps of:

- (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and
- (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

Claim 102 (canceled)

Claim 103 (currently amended): A method for identifying a polypeptide having a pectate lyase activity comprising the following steps:

- (a) providing a polypeptide as set forth in claim [[59]] 55;
- (b) providing a pectate lyase substrate; and
- (c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a pectate lyase activity.

Claims 104 to 105 (canceled)

Claim 106 (previously presented): A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

- (a) providing a polypeptide as set forth in claim 55;
- (b) providing a test compound;
- (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

Claim 107 (previously presented): A method for identifying a modulator of a pectate lyase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 55;
(b) providing a test compound;
(c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the pectate lyase, wherein a change in the pectate lyase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the pectate lyase activity.

Claims 108 to 110 (canceled)

Claim 111 (previously presented): A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, or a computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 55, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claims 112 to 120 (canceled)

Claim 121 (previously presented) A method for isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample comprising the steps of:

(a) providing an amplification primer sequence pair as set forth in claim 31 or claim 33;
(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,
(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample.

Claim 122 (canceled)

Claim 123 (previously presented) A method for isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample comprising the steps of:

- (a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof;
- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);
- (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and
- (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample.

Claims 124 to 125 (canceled)

Claim 126 (previously presented) A method of generating a variant of a nucleic acid encoding a polypeptide with a pectate lyase activity comprising the steps of:

- (a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and
- (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

Claims 127 to 135 (canceled)

Claim 136 (previously presented) A method for modifying codons in a nucleic acid encoding a polypeptide with a pectate lyase activity to increase its expression in a host cell, the method comprising the following steps:

- (a) providing a nucleic acid encoding a polypeptide with a pectate lyase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in

coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

Claim 137 (previously presented) A method for modifying codons in a nucleic acid encoding a pectate lyase polypeptide, the method comprising the following steps:

- (a) providing a nucleic acid encoding a polypeptide with a pectate lyase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a pectate lyase.

Claims 138 to 140 (canceled)

Claim 141 (currently amended): A method for producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:

- (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO: [[1]] 77, SEQ ID NO:131 or SEQ ID NO: [[7]] SEQ ID NO:133, or a subsequence thereof, and the nucleic acid encodes a pectate lyase active site or a pectate lyase substrate binding site;
- (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,
- (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites.

Claims 142 to 144 (canceled)

Claim 145 (previously presented) A method for making a small molecule comprising the following steps:

- (a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a pectate lyase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;
- (b) providing a substrate for at least one of the enzymes of step (a); and
- (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

Claim 146 (currently amended) A method for modifying a small molecule comprising the following steps:

- (a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24;
- (b) providing a small molecule; and
- (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the pectate lyase enzyme, thereby modifying a small molecule by a pectate lyase enzymatic reaction.

Claims 147 to 150 (canceled)

Claim 151 (currently amended) A method for determining a functional fragment of a pectate lyase enzyme comprising the steps of:

- (a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and
- (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a pectate lyase activity, thereby determining a functional fragment of a pectate lyase enzyme.

Claim 152 (canceled)

Claim 153 (previously presented) A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

- (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;
- (b) culturing the modified cell to generate a plurality of modified cells;
- (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

Claims 154 to 157 (canceled)

Claim 158 (currently amended): An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43 or 1 to 44, of ~~SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102,~~

~~SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134.~~

Claim 159 to 163 (canceled)

Claim 164 (previously presented) A method of increasing thermotolerance or thermostability of a pectate lyase, the method comprising glycosylating a pectate lyase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the pectate lyase.

Claim 165 (previously presented): A method for overexpressing a recombinant pectate lyase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

Claim 166 (previously presented) A method of making a transgenic plant comprising the following steps:

- (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell;
- (b) producing a transgenic plant from the transformed cell.

Claims 167 to 168 (canceled)

Claim 169 (previously presented) A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:

- (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24;
- (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

Claim 170 (currently amended) A method for hydrolyzing, breaking up or disrupting a pectin-₂ [[or]] pectate (polygalacturonic acid)-homogalacturonan-, rhamnogalacturonan- or plant cell wall-comprising composition comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing a composition comprising a pectin, [[or]] a pectate, a homogalacturonan, a rhamnogalacturonan or a plant cell well; and
- (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide hydrolyzes, breaks up or disrupts the pectin-₂ [[or]] pectate-, homogalacturonan-, rhamnogalacturonan- or plant cell wall-comprising composition, optionally wherein the composition comprises a bacterial cell wall, optionally wherein the plant is a cotton plant, a hemp plant or a flax plant.

Claims 171 to 172 (canceled)

Claim 173 (currently amended) A method for liquefying or removing a pectin, [[or]] a pectate (polygalacturonic acid) a homogalacturonan, a rhamnogalacturonan or a plant cell wall from a composition comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing a composition comprising a pectin, [[or]] pectate (polygalacturonic acid) a homogalacturonan, a rhamnogalacturonan or a plant cell wall; and
- (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide removes or liquefies the pectin, [[or]] pectate (polygalacturonic acid), homogalacturonan, rhamnogalacturonan or plant cell wall.

Claim 174 (currently amended) A detergent composition comprising a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the polypeptide has a pectate lyase activity.

Claims 175 to 176 (canceled)

Claim 177 (currently amended) A method for washing an object comprising the following steps:

- (a) providing a composition comprising a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing an object; and
- (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

Claim 178 (currently amended) A fiber, thread, textile, [[or]] fabric, or cellulosic material comprising a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claim 179 (currently amended) A method for fiber, thread, textile, [[or]] fiber or cellulosic scouring comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing a fiber, a thread, a textile, [[or]] a fabric, a cellulosic material; and
- (c) contacting the polypeptide of step (a) and the fiber, thread, textile, [[or]] fabric or cellulosic material of step (b) under conditions wherein the pectate lyase can scour the fiber, thread, textile, [[or]] fiber or cellulosic material,
optionally further comprising addition of an alkaline and thermostable amylase in the contacting of step (c),
optionally further comprising addition of a bleaching step.

optionally wherein the pectate lyase is an alkaline active and thermostable pectate lyase,

optionally further comprising a desizing treatment,

optionally wherein the desizing and scouring treatments are combined in a single bath,

optionally wherein the desizing or scouring treatments comprise conditions of between about pH 4.0 to pH 12.0 and temperatures from about 20°C to about 95°C, between about pH 8.0 to pH 10.0 and temperatures from about 37°C to about 95°C, or between about pH 8.5 to pH 9.5 and temperatures from about 70°C to about 95°C,

optionally wherein the desizing, scouring and bleaching treatments are done simultaneously or sequentially in a single-bath container,

optionally wherein the bleaching treatment comprises hydrogen peroxide or at least one peroxy compound which can generate hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator,

optionally wherein the cellulosic material comprises a crude fiber, a yarn, a woven or knit textile, a cotton, a denim, a wool, a natural fiber, a synthetic fiber, a linen, a flax, a ramie, a rayon, a hemp, a jute or a blend of natural and synthetic fibers.

Claims 180 to 189 (canceled)

Claim 190 (currently amended) A feed or a food comprising a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claim 191 (currently amended) A method improving the extraction of oil from an oil-rich plant material comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing an oil-rich plant material; and
- (c) contacting the polypeptide of step (a) and the oil-rich plant material.

Claims 192 to 193 (canceled)

Claim 194 (currently amended) A method for preparing a fruit or vegetable juice, syrup, puree or extract comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing a composition or a liquid comprising a fruit or vegetable material; and
- (c) contacting the polypeptide of step (a) and the composition, thereby preparing the fruit or vegetable juice, syrup, puree or extract.

Claim 195 (currently amended) A paper, [[or]] paper product, [[or]] paper pulp, wood, wood product, wood pulp or cellulosic material comprising a pectate lyase as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claim 196 (currently amended) A method for treating a paper, [[or]] a paper product, a wood, a wood product, a [[or]] wood pulp or a cellulosic material comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing a composition comprising a paper, [[or]] a paper product, a wood, a wood product, a [[or]] wood pulp or a cellulosic material; and
- (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the pectate lyase can treat the paper, [[or]] a paper product, a wood, a wood product, a [[or]] wood pulp or a cellulosic material.

Claim 197 (currently amended) A pharmaceutical composition comprising a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claim 198 (canceled)

Claim 199 (currently amended) An oral care product comprising a polypeptide as set forth in claim [[59]] 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

Claim 200 (canceled)

Claim 201 (previously presented) A method for ameliorating soft-rot spoilage in a plant or plant part comprising administering a composition that decreases the expression or activity of a pectate lyase in the plant or plant part, wherein the composition comprises an antibody as set forth in claim 95, or an antisense oligonucleotide, a ribozyme or an RNAi comprising an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

Claim 202 (canceled)

Claim 203 (previously presented) A method for slowing the normal growth of the powdery mildew pathogen Erysiphe cichoracearum in a plant or plant part comprising administering a composition that decreases the expression or activity of a pectate lyase in the plant or plant part, wherein the composition comprises an antibody as set forth in claim 95, or an antisense oligonucleotide, a ribozyme or an RNAi comprising an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

Claim 204 (currently amended): An isolated, synthetic or recombinant nucleic acid encoding a polypeptide having pectate lyase (EC 4.2.2.2) activity ~~having a sequence~~ comprising a sequence modification of SEQ ID NO:131, wherein the modification of SEQ ID NO:131 comprises one or more of the following changes:

- (a) the nucleotides at residues 352 to 354 are CAT or CAC,
- (b) the nucleotides at residues 544 to 546 are GTG, GTT, GTC, or GTA,
- (c) the nucleotides at residues 568 to 570 are TTG, TTA, CTT, CTC, CTA, or CTG,
- (d) the nucleotides at residues 589 to 591 are GGT, GGC, GGA, or GGG,
- (e) the nucleotides at residues 622 to 624 are AAG or AAA,
- (f) the nucleotides at residues 655 to 657 are ATG,

(g) the nucleotides at residues 667 to 669 are GAG or GAA,
(h) the nucleotides at residues 763 to 765 are CGG, CGT, CGC, CGA, AGA, AGG,
(i) the nucleotides at residues 787 to 789 are AAG or AAA,
(j) the nucleotides at residues 823 to 825 are TAT or TAC,
(k) the nucleotides at residues 925 to 927 are TGG, or
(l) the nucleotides at residues 934 to 936 are GTT, GTG, GTC, or GTA,
wherein optionally the nucleic acid lacks a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof; or

wherein optionally the nucleic acid of further comprising a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme.

Claim 205 (currently amended): An isolated, synthetic or recombinant nucleic acid encoding a polypeptide having a pectate lyase (EC 4.2.2.2) activity: having a sequence

(a) comprising a nucleic acid sequence comprising a sequence modification of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID

~~NO:125, SEQ ID NO:127 or SEQ ID NO:129~~, wherein the sequence modification comprises one or more of the following changes:

- (a) the nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 are changed to CAT or CAC,
- (b) the nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 are changed to GTG, GTT, GTC, or GTA,
- (c) the nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 are changed to TTG, TTA, CTT, CTC, CTA, or CTG,
- (d) the nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 are changed to GGT, GGC, GGA, or GGG,
- (e) the nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 are changed to AAG or AAA,
- (f) the nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 are changed to ATG,
- (g) the nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 are GAG or GAA,
- (h) the nucleotides at the equivalent of residues 763 to 765 of SEQ ID NO:131 are changed to CGG, CGT, CGC, CGA, AGA, AGG,
- (i) the nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 are changed to AAG or AAA,
- (j) the nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 are changed to TAT or TAC,
- (k) the nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 are changed to TGG, or
- (l) the nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 are changed to GTT, GTG, GTC, or GTA[.],
 - wherein optionally the nucleic acid sequence lacks a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof; or
 - wherein optionally the nucleic acid sequence further comprises a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a

carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme

Claims 206 to 207 (canceled)

Claim 208 (currently amended): An isolated, synthetic or recombinant polypeptide having pectate lyase (EC 4.2.2.2) activity comprising an amino acid sequence comprising a sequence modification of SEQ ID NO:132, wherein the modification of SEQ ID NO:132 comprises one or more of the following mutations: the alanine at amino acid position 118 is histidine, the alanine at amino acid position 182 is valine, the threonine at amino acid position 190 is leucine, the alanine at amino acid position 197 is glycine, the serine at amino acid position 208 is lysine, the threonine at amino acid position 219 is methionine, the threonine at amino acid position 223 is glutamic acid, the serine at amino acid position 255 is arginine, the serine at amino acid position 263 is lysine, the asparagine at amino acid position 275 is tyrosine, the tyrosine at amino acid position 309 is tryptophan, or, the serine at amino acid position 312 is valine,

wherein optionally the polypeptide lacks a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof; or

wherein optionally the polypeptide comprises a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme.

Claim 209 (currently amended): An isolated, synthetic or recombinant polypeptide having a pectate lyase (EC 4.2.2.2) activity and comprising an amino acid having a sequence comprising a

sequence modification of ~~SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130~~, wherein the sequence modification comprises one or more of the following changes:

- (a) the amino acid at the equivalent of the alanine at residue 118 of SEQ ID NO:132 is changed to a histidine,
- (b) the amino acid at the equivalent of the alanine at residue 182 of SEQ ID NO:132 is changed to a valine,
- (c) the amino acid at the equivalent of the threonine at residue 190 of SEQ ID NO:132 is changed to a leucine,
- (d) the amino acid at the equivalent of the alanine at residue 197 of SEQ ID NO:132 is changed to a glycine,
- (e) the amino acid at the equivalent of the serine at residue 208 of SEQ ID NO:132 is changed to a lysine,
- (f) the amino acid at the equivalent of the threonine at residue 219 of SEQ ID NO:132 is changed to a methionine,
- (g) the amino acid at the equivalent of the threonine at residue 223 of SEQ ID NO:132 is changed to a glutamic acid,
- (h) the amino acid at the equivalent of the serine at residue 255 of SEQ ID NO:132 is changed to a arginine,
- (i) the amino acid at the equivalent of the serine at residue 263 of SEQ ID NO:132 is changed to a lysine,

(j) the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine,

(k) the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or,

(l) the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine[[.]].

wherein optionally the polypeptide sequence lacks a signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, or a combination thereof; or

wherein optionally the polypeptide sequence further comprises a heterologous sequence, wherein optionally the heterologous sequence comprises a heterologous signal sequence, a carbohydrate binding module, a pectin methyl esterase domain, a catalytic domain, a prepro domain, an enzyme or a combination thereof, and optionally wherein the heterologous signal sequence, carbohydrate binding module, pectin methyl esterase domain, catalytic domain, prepro domain, enzyme or a combination thereof, is derived from another pectate lyase or a non-pectate lyase enzyme.

Claims 210 to 211 (canceled)

Claim 212 (currently amended): A method for generating a modified pectate[[-]]_lyase-encoding nucleic acid comprising making one or more sequence modifications to a pectate[[-]]_lyase-encoding nucleic acid, wherein the changes in the pectate[[-]]_lyase-encoding nucleic acid are equivalent to one or more of the following:

(a) changing nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 to CAT or CAC,

(b) changing nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 to GTG, GTT, GTC, or GTA,

(c) changing nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 to TTG, TTA, CTT, CTC, CTA, or CTG

(d) changing nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 to GGT, GGC, GGA, or GGG,

(e) changing nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 to AAG or AAA,

(f) changing nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 to ATG,

(g) changing nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 to GAG or GAA,

(h) the nucleotides at the equivalent of residues 763 to 765 of SEQ ID NO:131 to CGG, CGT, CGC, CGA, AGA, AGG,

(i) changing nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 to AAG or AAA,

(j) changing nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 to TAT or TAC,

(k) changing nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 to TGG, or

(l) changing nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 to GTT, GTG, GTC, or GTA.

Claim 213 (canceled)

Claim 214 (currently amended): The method of claim 212, wherein the pectate-lyase encoding nucleic acid comprises a nucleic acid having a sequence as set forth in ~~SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID~~

~~NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133.~~

Claim 215 (previously presented): A method for generating a modified pectate lyase comprising making one or more sequence modifications to a pectate lyase, wherein the changes in the pectate lyase are equivalent to one or more of the following changes:

- (a) the amino acid at the equivalent of the alanine at residue 118 of SEQ ID NO:132 is changed to a histidine,
- (b) the amino acid at the equivalent of the alanine at residue 182 of SEQ ID NO:132 is changed to a valine,
- (c) the amino acid at the equivalent of the threonine at residue 190 of SEQ ID NO:132 is changed to a leucine,
- (d) the amino acid at the equivalent of the alanine at residue 197 of SEQ ID NO:132 is changed to a glycine,
- (e) the amino acid at the equivalent of the serine at residue 208 of SEQ ID NO:132 is changed to a lysine,
- (f) the amino acid at the equivalent of the threonine at residue 219 of SEQ ID NO:132 is changed to a methionine,
- (g) the amino acid at the equivalent of the threonine at residue 223 of SEQ ID NO:132 is changed to a glutamic acid,
- (h) the amino acid at the equivalent of the serine at residue 255 of SEQ ID NO:132 is changed to a arginine,
- (i) the amino acid at the equivalent of the serine at residue 263 of SEQ ID NO:132 is changed to a lysine,
- (j) the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine,
- (k) the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or,
- (l) the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine.

Claim 216 (currently amended): The method of claim 215, wherein the pectate lyase comprises a sequence as set forth in ~~SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130-SEQ ID NO:132 or SEQ ID NO:134.~~

Claim 217 (canceled)

Claim 218 (currently amended): A formulation for treating a material with a pectate lyase comprising a pectate lyase as set forth in claim [[59]] 55, wherein the formulation comprises a dosage of pectate lyase between about 0.1 ml to 1 ml per kg of material in the range of between about 1 gram per ton per ton treated material and 100 or more grams per ton per ton treated material

optionally wherein the material comprises a paper, a paper product, a paper pulp, a wood, a wood product, a wood pulp, a fiber, a thread, a textile, a fabric, or a cellulosic material.

Claim 219 (currently amended): The formulation of claim 218, wherein the dosage is between about 1 gram per ton treated material and 100 or more grams per ton treated material, between about 10 grams per ton and 90 grams per ton, between about 20 grams per ton and 80 gram per ton, between about 30 grams per ton and 70 grams per ton, or between about 40 grams per ton and 50 grams per ton.

Claim 220 (previously presented): The formulation of claim 218, wherein the dosage is between about 1 μ g per gram and 100 or more μ g per gram, between about 10 μ g per gram and 90 μ g per gram, between about 20 μ g per gram and 80 μ g per gram, between about 30 μ g per gram and 70 μ g per gram, or between about 40 μ g per gram and 50 μ g per gram.

Claim 221 (previously presented): The formulation of claim 218, wherein the dosage is between about 0.5 mg per pound and 50 or more mg per pound, between about 1 mg per pound and 45 mg per pound, between about 5 mg per pound and 40 mg per pound, between about 10 mg per pound and 35 mg per pound or between about 15 mg per pound and 30 mg per pound.

Claim 222 (canceled)

Claim 223 (previously presented): The formulation of claim 218, wherein the formulation is a water-based formulation.

Claim 224 (currently amended): The formulation of claim 223, wherein the dosage formulation comprises an enzyme strength of between about 500 to 30,000 units/ml.

Claim 225 (currently amended): The formulation of claim 224, wherein the dosage formulation comprises an enzyme strength of between about 1000 to 25,000 units/ml, 1000 to 20,000 units/ml, 1000 to 15000 units/ml, 1000 to 10,000 units/ml, between about 1000 to 5000 units/ml, between about 2000 to 20000 units/ml, between about 2000 to 10000 units/ml, or between about 2000 to 5000 units/ml.

Claim 226 (currently amended): The formulation of claim 225, wherein the dosage formulation comprises an enzyme strength of about [[1000]] 6000 units/ml or about 8000 units/ml.

Claim 227 (canceled)

Claim 228 (canceled)

Claim 229 (previously presented): The formulation of claim 218, further comprising a glycerol, sucrose, sodium chloride, dextrin, propylene glycol, sorbitol, sodium sulphate or TRIS, or an equivalent.

Claim 230 (canceled)

Claim 231 (currently amended): The formulation of claim 218, wherein the buffer formulation comprises pH 7, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 35% glycerol, 300 ppm proxel; pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 10% sodium chloride, 25% glycerol, 300 ppm proxel; pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 5.5, 35% glycerol, 300 ppm proxel; pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; or, 20 mM acetate buffer, pH 5.5, 35% glycerol; 20 mM MOPS, pH 7 or 25 mM MOPS, 50 mM NaCl, pH 7.5; pH 5.0, 40 mM TRIS; pH 7.0, 40 mM TRIS; pH 8.0, 40 mM TRIS; pH 7.5, 50% glycerol; pH 7.5, 20% NaCl; pH 7.5, 30% propylene glycol; pH 7.5, 100 mM sodium sulfate; pH 5.5, 35% glycerol; or, any combination thereof, or, equivalents thereof.

Claim 232 (currently amended): A bioscouring process comprising the following steps:

- (a) providing a pectate lyase as set forth in claim [[59]] 55;
- (b) providing a pectin-₂ [[or]] polygalacturonic acid-(pectate-), homogalaturonan, rhamnogalacturonan- or plant cell wall-comprising material;
- (c) contacting the pectate lyase of (a) with the material of (b) optionally under conditions comprising
 - (i) between about pH 8.5 to about pH 10.0,
 - (ii) a [[in]] bicarbonate buffer,
 - (iii) comprising a non-ionic wetting agent at, about 1 g/L,
 - (iv) a liquor where the pectate lyase ratio in an enzyme bath is between about 10:1 to 50:1 L pectate lyase:kg of material,
 - (v) a where the pectate lyase dose is between about 0.1 and [[0.2]] 1 ml of a concentrated extract per kg of material, or equivalent,
 - (vi) [[at]] a temperature range between about 50°C to 70°C to about 95°C,

(vii) [[and]] a treatment time of about 20 min, or

(viii) a combination of any of (i) through (vii);

optionally further comprising at least one rinse cycle,

optionally wherein the conditions comprise between about pH 4.0 to pH 12.0 and temperatures from about 20°C to about 95°C, between about pH 8.0 to pH 10.0 and temperatures from about 37°C to about 95°C, or between about pH 8.5 to pH 9.5 and temperatures from about 70°C to about 95°C,

optionally wherein the dosage is between about 1 gram per ton treated material and 100 or more grams per ton treated material, between about 10 grams per ton and 90 grams per ton, between about 20 grams per ton and 80 gram per ton, between about 30 grams per ton and 70 grams per ton, or between about 40 grams per ton and 50 grams per ton,

optionally wherein the dosage is between about 1 µg per gram and 100 or more µg per gram, between about 10 µg per gram and 90 µg per gram, between about 20 µg per gram and 80 µg per gram, between about 30 µg per gram and 70 µg per gram, or between about 40 µg per gram and 50 µg per gram,

optionally wherein the dosage is between about 0.5 mg per pound and 50 or more mg per pound, between about 1 mg per pound and 45 mg per pound, between about 5 mg per pound and 40 mg per pound, between about 10 mg per pound and 35 mg per pound or between about 15 mg per pound and 30 mg per pound,

optionally wherein the contacting step further comprises use of a chelant, a dispersant, a surfactant, or a combination thereof,

wherein optionally the chelant is excluded from the enzyme bath and is added after about 20 minutes of enzyme treatment and retained for about 10 minutes before discharging bath.

Claim 233 (currently amended): The bioscouring process of claim 232, wherein the material comprises a paper, a paper product, a paper pulp, a wood, a wood product, a wood pulp, a fiber, a thread, a fabric, a textile or a cloth cellulosic material.

Claims 234 to 235 (canceled)

Claim 236 (new): The isolated, synthetic or recombinant polypeptide of claim 55, wherein the pectate lyase activity is exo-acting or endo-acting.

Claim 237 (new): The isolated, synthetic or recombinant polypeptide of claim 55, wherein the pectate lyase activity is thermostable or thermotolerant.

Claim 238 (new): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the pectate lyase activity is exo-acting or endo-acting.

Claim 239 (new): The isolated, synthetic or recombinant nucleic acid of claim 1, wherein the pectate lyase activity is thermostable or thermotolerant.

Claim 240 (new) The isolated, synthetic or recombinant nucleic acid of claim 16, wherein the activity comprises pectin lyase (EC 4.2.2.10) activity, polygalacturonase (EC 3.2.1.15) activity, exo-polygalacturonase (EC 3.2.1.67) activity, exo-polygalacturonate lyase (EC 4.2.2.9) activity and/or exo-poly-alpha-galacturonosidase (EC 3.2.1.82) activity.

Claim 241 (new) The isolated, synthetic or recombinant polypeptide of claim 68, wherein the activity comprises pectin lyase (EC 4.2.2.10) activity, polygalacturonase (EC 3.2.1.15) activity, exo-polygalacturonase (EC 3.2.1.67) activity, exo-polygalacturonate lyase (EC 4.2.2.9) activity and/or exo-poly-alpha-galacturonosidase (EC 3.2.1.82) activity.